Kalirin Decreases Bone Mass Through Effects in Both Osteoclasts and Osteoblasts

Su Huang[1], Pierre Eleniste[1], Paula LeBlanc[2], Drew Brown[2], Matthew Allen[2], Angela Bruzzaniti[1,2]

[1]Department of Oral Biology, Indiana University School of Dentistry and [2]Department of Anatomy and Cell Biology, Indiana Universtiy School of Medicine

Indiana University – Purdue University Indianapolis

Abstract

Bone homeostasis is maintained by the balance between osteoclasts which degrade bone and osteoblasts, which form new bone. When the activity of either of these cells is dysregulated, bone loss can ensue, leading to osteoporosis, a disease characterized by low bone mass and an increase in bone fragility and risk of fracture. The activity of osteoclasts and osteoblasts is regulated by local and systemic factors, as well as by key signaling proteins expressed in these cells. Kalirin is a novel GTP-exchange factor protein that plays a role in signaling pathways leading to cytoskeletal remodeling and dendritic spine formation in neurons, but its function in other cells is unknown. Western blotting and real time PCR confirmed that Kalirin is expressed in osteoclasts and osteoblasts, suggesting it may play a role in regulating bone cell function and bone mass. We used micro-CT to examine the bone phenotype of 14 week old female mice lacking Kalirin in all tissues (Kal-KO). Kal-KO mice exhibited a 40% lower trabecular bone volume in the distal femur compared to wild-type (WT) mice (n=9/group, $p<0.05$). We next quantified osteoclasts in histological sections by counting multinucleated cells expressing tartrate-resistant acid phosphatase (TRAP), a marker of mature osteoclasts. We found 48% higher osteoclast surface/bone surface in trabecular bone of Kal-KO mice, compared to WT mice (n=6/group, $p<0.05$). Osteoclast differentiation is controlled by osteoblasts, which secrete receptor activator of NF-kB ligand (RANKL), macrophage colony stimulating factor (MCSF) and osteoprotegerin (OPG), a decoy receptor for RANKL. We examined if Kalirin could regulate osteoclast differentiation *in vitro*. Osteoclasts were generated from the bone marrow of WT or Kal-KO mice by incubation with RANKL and MCSF for 7 days, and TRAP+ multinucleated cells were counted. Consistent with our in vivo studies, osteoclast number was significantly higher in cultures from Kal-KO mice, compared to WT mice. We next examined if Kalirin altered the ratio of secreted RANKL and OPG secreted by osteoblasts. Osteoblasts were generated from the calvaria of 2 day old neonates and the level of secreted RANKL and OPG in conditioned media was quantified by ELISA. Consistent with increased osteoclast differentiation, we found a higher RANKL/OPG ratio in conditioned media from Kal-KO osteoblasts, compared to WT cells. These data confirm a role for Kalirin in the regulation of trabecular bone mass through effects in both osteoclasts and osteoblasts.